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Evaluation of Anti-Candida potential of Indigenous Plants and Herbs

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Abstract : *Candida*, a yeast is the common cause of fungal infection in humans. It has almost 150 strains from which about 50% of all infections are caused by *Candida albicans*, but there are atleast four other pathogenic species of this fungus, namely *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. tropicalis*. In this study antifungal activity and minimum inhibitory concentrations (MICs) of the ethanolic and aqueous extracts from fifteen plants, namely *Acacia nilotica* (Babul), Allium Cepa (Onion), Allium Sativum (Garlic), Azadirachtaindica (Neem), Cinnamomumverum (Cinnamon), Curcuma longa (Turmeric), Citrus Limon, Lantana Camara (Wild-sage), Lawsoniainermis (Heena), *Ocimum sanctum* (Basil), Piper Nigrum (Pepper), *Pithecellobium dulce* (Jungle Jalebi), *Syzygium aromaticu* (Clove), *Zingiber officinale* (Ginger), Ziziphus mauritiana (Ber) were tested against five species of *Candida* viz. *C. albicans, C. tropicales, C. krusei, C. parapsilosis* and *C. glabrata*. Of all tested extracts, Cinnamon (C.verum) was found to be most promising as it inhibited the growth of all tested *Candida*species. Apart from (C.verum), *S.aromaticum* and *O. sanctum* also gave good results with some species. Further in MIC study, a minimum concentration of 25µg of cinnamon was found to be most optimum for all five strains.
Key words : *Candida,* Antimycoticactivity, *Cinnamomum verum, C. tropicalis, C. albicans.*

Introduction:

Candida, a genus of yeast, is the most common cause of opportunistic fungal infections worldwide[1]. Many species are harmless commensals or endosymbionts including *C. albicans* in many hosts and humans; however, when mucosal barriers are disrupted or the immune system is compromised they can invade and cause disease[2]. Antibiotics promote yeast infections, including gastrointestinal *Candida* overgrowth, and penetration of the GI mucosa[3]. While women are more susceptible to genital yeast infections, men can also be infected. Some factors, such as prolonged antibiotic use, increase the risk for both men and women. People with diabetes or impaired immune systems such as those with HIV, are more susceptible to yeast infections[4]. Systemic infections of the bloodstream and major organs (candidemia or invasive candidiasis), especially in immune-compromised patients, affect over 90,000 people a year in the U.S.[5].

The DNA of several *Candida* species has been sequenced and almost 150 different species are known. Of all *Candida,* five are frequently encountered with human infections.

Candida albicans, a dimorphic fungus, cause the infection candidiasis or thrush in humans. *C. albicans* is responsible for 50–90% of all cases of candidiasis in humans[6]. It is the most common cause of vaginal candidiasis, oral thrush, intestinal condidiasis and life-threatening systemic candidiasis[7]. Systemic fungal

infections (fungemias) including those by *C. albicans* have emerged as on important cause of morbidity and mortality in immuno-compromised patients.

Candida glabrata, a non-dimorphic haploid yeast of the genus *Candida,* was thought to be a primarily non-pathogenic organism until recently. However, with the ever increasing population of immune-compromised individuals, trends have shown *C. glabrata* to be a highly opportunistic pathogen of the urogenital tract, and of the bloodstream[8].

Candida krusei is an emerging fungal nosocomial pathogen primarily found in the immune-compromised and those with hematological malignancies. It has natural resistance to fluconazole which is a standard antifungal agent. It is most often found in patients who have had prior fluconazole exposure, sparking debate and conflicting evidence as to whether fluconazole should be used prophylactically[9].

Candida parapsilosis is a fungal species of the yeast family that has become a significant cause of sepsis, wound and tissue infections in immuno-compromised patients.*C. parapsilosis* is not an obligate human pathogen, have been isolated from nonhuman sources like domestic animals, insects or soil. It is one of the fungi most frequently isolated from the human hands. Immuno-compromised individuals and surgical patients, particularly those having surgery of the gastrointestinal tract are at very high risk for infection with *C. parapsilosis*[10].

Candida tropicalis has been identified as the most prevalent pathogenic yeast species of the Candida-non-albicans group. Historically, *C. albicans* has been the major species responsible for causing candidiasis in immune-compromised and immunocompetent patients. However, infections like candidiasis due to *C. tropicalis* have increased dramatically on a global scale thus proclaiming this organism to be an emerging pathogenic yeast. The reasons for this organism's dominance and its resistance to fluconazole have been difficult to elucidate. In addition the mechanism of this organism's pathogenicity and the consequent immune response remain to be clarified[11].

Methodology:

Sample collection:

Fresh leaves of *Acacia nilotica* (Babul), *Azadirachta indica* (Neem), *Lantana camara* (Wild-sage), *Lawsonia inermis* (Heena), *Ocimum sanctum* (Tulsi), *Pithecellobium dulce* (Jungle jalebi),*Ziziphus mauritiana* (Ber) were collected from the garden of Amity University and its surroundings. *Allium cepa* (Onion), *Allium sativum* (Garlic), *Cinnamomum verum* (Cinnamon), *Curcuma longa* (Turmeric),*Citrus limon* (Lemon), *Piper nigrum* (Pepper), *Syzygium aromaticum*(Clove), *Zingiber officinale* (Ginger)were procured from local market.

Preparation of plant extracts:

All plant samples were washed thoroughly, first with tap water (two to three times) and then with distilled water to remove the dust particles. For preparation of plant extract, 10gm of each sample was divided into two parts and crushed into very fine paste using double distilled water and Methanol. These pastes were transferred into tarson tubes, and kept in a dark and hygienic place for 24 hrs. The aqueous (aq.)and methanolic (met.) extracts were filtered and dried. The dried extract was weighed, dissolved in four times volume of DMSO and carefully collected in eppendorf tubes. These tubes were kept in the freezer for future use.

Test organisms:

Five organisms*: C. albicans, C. tropicales, C. krusei, C. paraplilosie, C. glabrata* were included in the study. The organisms were made as stock by mixing 100 µl of suspension in 10 ml of sterile Sabouraud dextrose broth and grown overnight. The organisms were maintained by sub culturing them on Sabouraud dextrose agar at regular intervals and usedthroughout the study.

Antimicrobial activity assay:

The microbial susceptibility test was done by using gel diffusion method[12]. SDA media was prepared as per the supplier's instruction and sterilized by autoclaving at 121°C for 15 min. 4-6 wells of 0.5 mm width and

0.5 mm depth were made at equal distance on each plate aseptically. Separate plates were inoculated with 50 µl of *C. albicans*, *C. tropicalis*, *C. krusei*, *C. parapsilosis* and *C. glabrata*. For each fungus, 50 µl of extract was loaded in the wells and plates were incubated at 37°C for 48 h. Antifungal (Antimycotic) activity of each extract was expressed in terms of zone of inhibition (mm). DMSO was used as negative control in each strain and antifungal agent fluconazole, Voriconazole and echinocandin were taken as positive control. Each experiment was repeated thrice and average of all values was taken.

Minimum inhibitory concentration (MIC)

MIC is the lowest concentration which results in the reduction of inoculums viability. A gel diffusion method was used with slight modifications to determine minimal inhibitory concentration (MIC) values and this test was done for methanolic extract of *C.verum*. Plant extracts were serially diluted, ranging from6.25 µg up to 100 µg from the crude extract. In each well, 50 µl of each extract was loaded. The petriplates were incubated for 48h at 37 °C with daily monitoring. All experiments were done in triplicate. The diameter of inhibition zone was measured in mm. MIC values were calculated by comparing growth in control wells and the extract blank, which consisted of uninoculated plates. The MIC of the extracts was defined as the lowest concentration of plant extract that caused growth inhibition of more than 90% at 48 h, as compared to the control.

Results and Discussion:

Fifteen locally available plants were taken for the study and their antifungal effect against five *Candida* species was studied. In this work, the objective was to discover a good alternative against the pathogenic fungi which was free of the disadvantages of synthetic anti-fungal agents[13]. After 48 hrs, the results were observed and described as inhibition zone (in mm). Figure 1 demonstrates zone of inhibition obtained against *C. albicans* in the different extracts of plants. *A. indica*(aq. and met.), *C. verum* (met.) and *O. sanctum* (met.) showed good result against *C. albicans*, with aqueous extract of *A. indica* giving maximum zone of inhibition. Figure 2 represents the activity pattern of extracts against *C. glabrata* with aqueous extract of *A.cepa* showing maximum potential whereas *S. aromaticum*(aq.) showed least activity. The activity pattern for *C. krusei* is given in figure 3. It shows maximum zone of inhibition with *C. verum* (met.). *C. parapsilosis* was found to be most resistant in our study with only two out of the fifteen test plants showing any effect on this strain and as evident in figure 4 only*C. verum*(met) and *S. aromaticum* (aq.) had significantly inhibited the growth. Zone of inhibition for *C.tropicalis*is represented in figure 5 where methanolic extract of *C. verum*, *S. aromaticum*, *L. inermis* and *A. nilotica* are showing good inhibition.

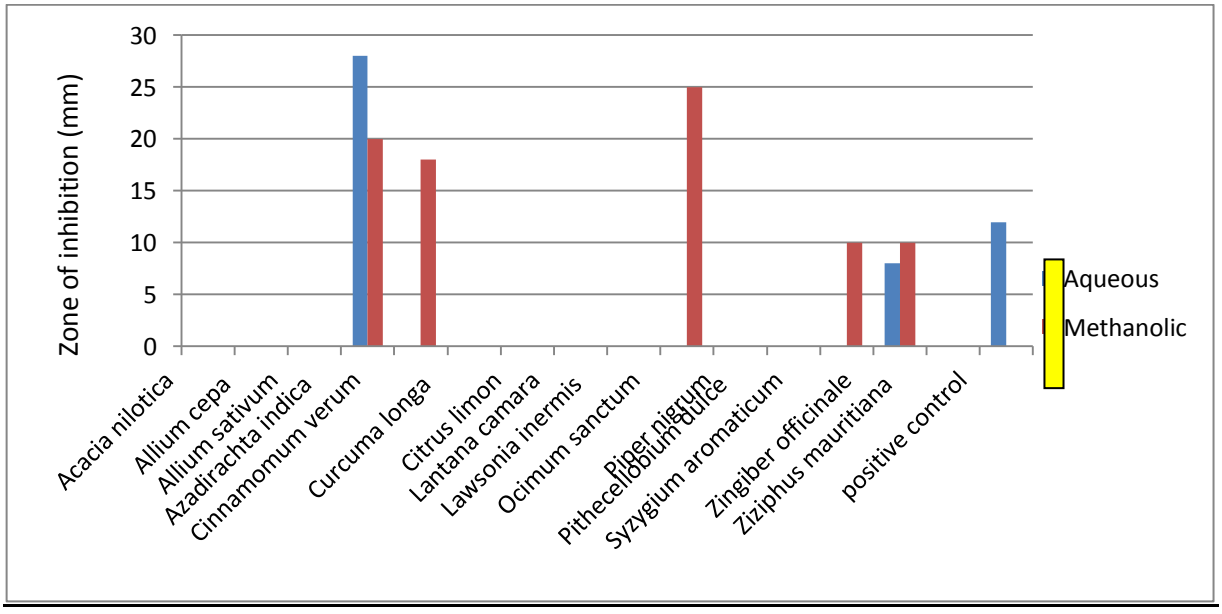

Figure 1. Inhibitory activity of test plants against *C.albicans*

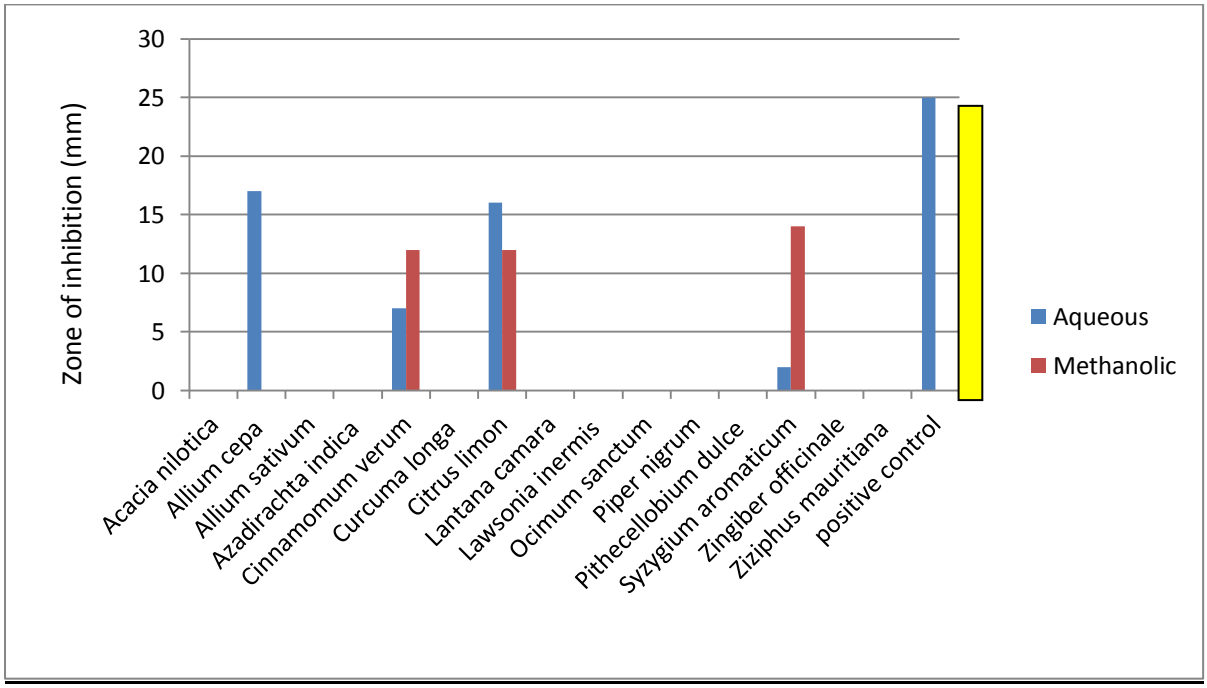

Figure 2. Inhibitory activity of test plants against *C.glabrata*

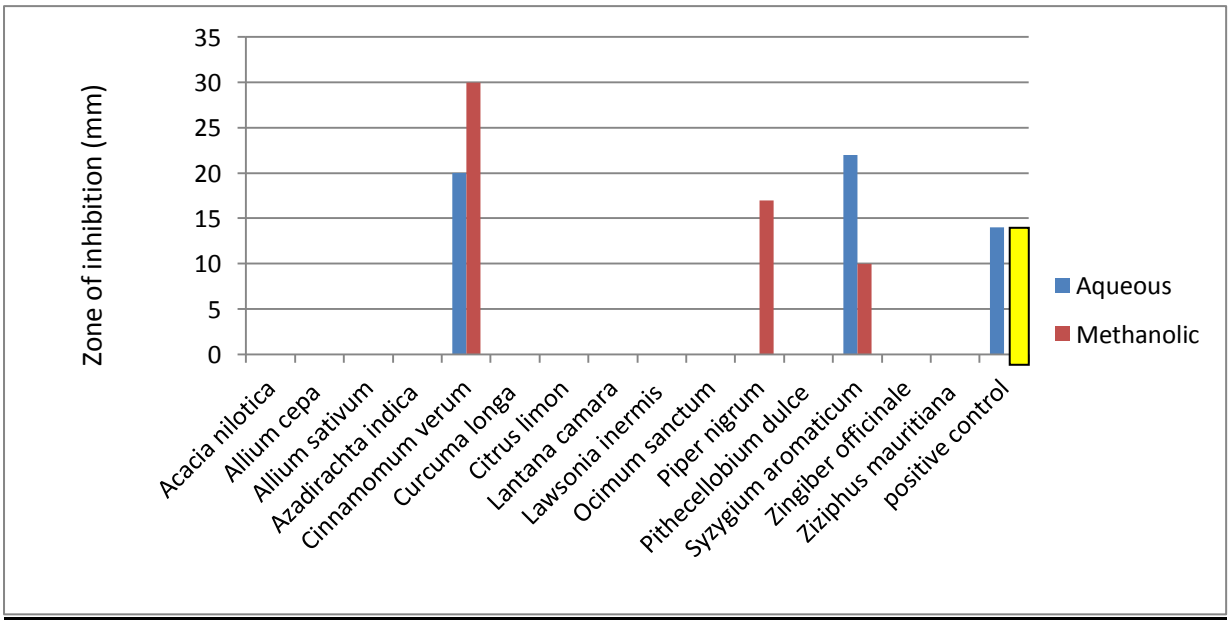

Figure 3. Inhibitory activity of test plants against *C.krusei*

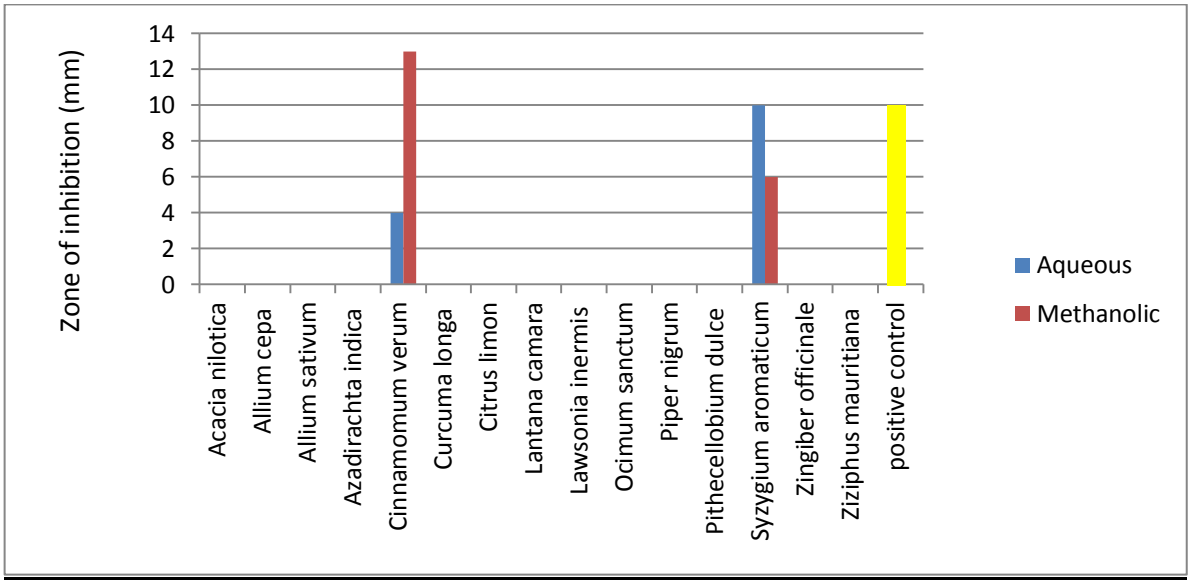

Figure 4. Inhibitory activity of test plants against *C.parapsilosis*

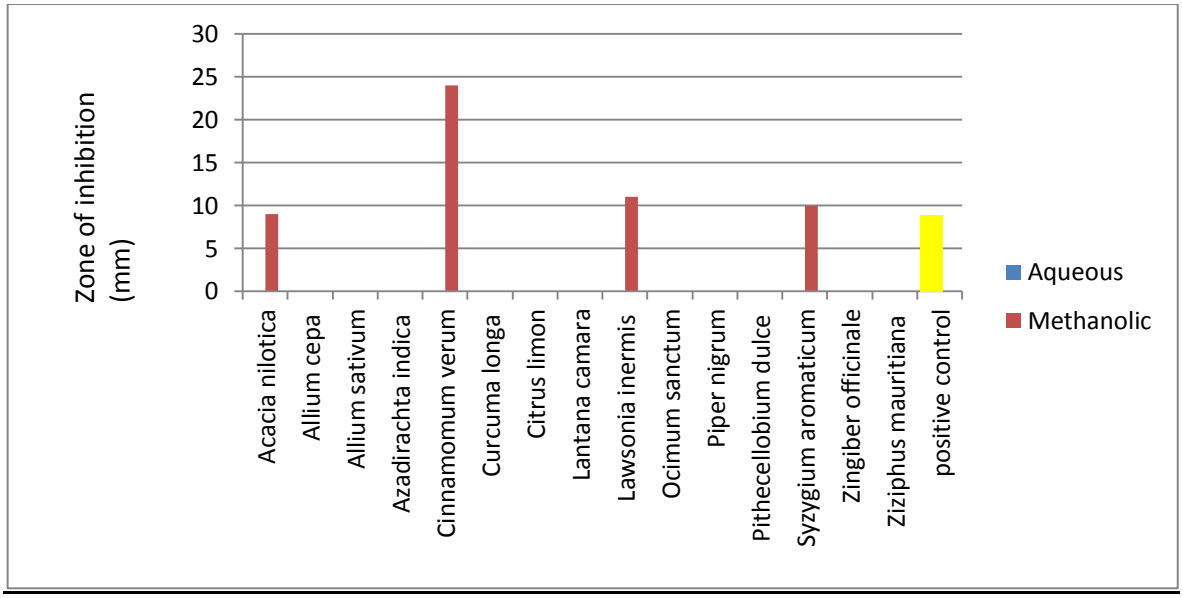

Figure 5. Inhibitory activity of test plants against *C.tropicalis*

The extracts from only two (2/15) plants, *C. verum* and *S. aromaticum* were found to be most potential as antifungal agent because all five strains of *Candida* were susceptible for these two plants. There was no significant zone of inhibition in *A. sativum, C. longa, L. camara, P. dulce* and *Z. mauritiana* against any of the fungal species. Antifungal activity of some medicinal plants extracts such as *C. verum* and *S. aromaticum* have also been reported by other workers[14].In the study conducted by Costa et al.(2015), aqueous and hydroalcoholic extracts of seven plants namely *Eugenia uniflora, Piper diospyrifolium, Piper hispidum, Psidium guajava, Rosmarinus officinalis, Senna spectabilis* and *Tetradenia riparia* were investigated for antimicrobial activity against *C. albicans, C. parapsilosis*and *C. tropicalis* and all plant species showed antimicrobial activity against these yeasts[15]. Some medicinal plants of Arabian peninsula, including *Rhamnusglobosa*, *Ocimumbasilicum*, *Tecomastans* and *Coleus forskohlii* have also been studied and the results showed high inhibitory growth in yeast after treatment with *R. globosa* and *O. basilicum*[16]. In a work done by Martins et al.(2014) the antifungal activity of extracts from ten different plants, commonly used in folk medicine, were evaluated against nineteen *Candida* strains,in which *Juglansregia* extract was very effective, exerting an inhibitory effect against all the tested *Candida* strains[6]. Recently *Cinnamomum zeylanicum* and *Melaleuca alternifolia* essential oil and honey have been reported as promising agents for oral candidiasis, therapy including in HIV positive patients as it showed good inhibitory activity against *Candida* strains[17].

As *C. verum* was found to be having inhibitory effect on all test strains of *Candida*, the MIC study was done with different concentrations of methanolic extracts of *C. verum*only. All five *Candida* strains were incubated with five (6.25, 12.5, 25, 50 and 100 µg) concentration of *C. verum* extract. The results were recorded after 48 hr and represented as zone of growth inhibition in mm (Figure 6). A minimum of 25 µg was found to be most optimum concentration which showed significant inhibition in all test strains of *Candida*. Rathod et al. (2015) conducted a study in which aqueous and acetone extracts of two plant species were screened *in vitro* for their antifungal activity against fungus *C. albicans*. 40µl concentration of Minimal Inhibitory Concentration (MIC) of Sandalwood extract in acetone and 60µl concentration of MIC of Arjun barks extract in acetone were found to be active[18].

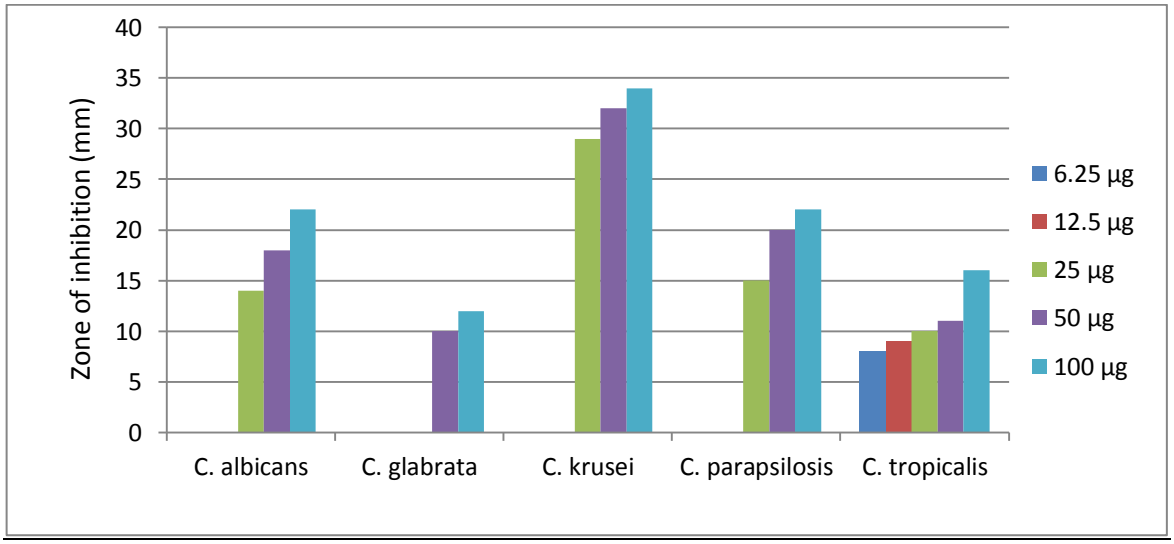

Figure 6: MIC study of methanolic extract of *Cinnamomum verum* for *Candida* strains

Conclusion:

The genus *Candida* has caused significant morbidity and mortality in human beings and invasive candidiasis remains a leading cause of mycosis related mortality. Though *C. albicans* remains the predominant cause, several less common species are emerging which exhibit resistance to triazoles or amphotericin B[19,20]. Therefore, non-pharmacologic preventive strategies should be emphasized[21,22].The results obtained from this work showed that plant extracts have potent antifungal effects against *Candida* species In particular aqueous and methanolic extracts of *C. verum* and *S. aromaticum* offer effective bioactive compounds for growth inhibition of the fungi. Even at lowest concentrations, these species showed antifungal activity nearly equal to that of the commercial fungicide used as a positive control. Further studies are needed to determine the chemical identity of the bioactive compounds responsible for the observed antifungal activity. Natural plant-derived fungicides may be a source of new alternative, in particular with antifungal activity[23,24,25].

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